A COMPARISON OF THE EFFECTS OF A CARBOHYDRATE MOUTH RINSE ON CYCLING PERFORMANCE IN DEPLETED AND NON-DEPLETED GLYCOGEN STATES

NICOLE HICKS & BAILEY SULLIVAN

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ABSTRACT:

Performance improvements have been reported when mouth rinsing with a carbohydrate (CHO) solution during short duration, high intensity endurance exercise. Limited research has been conducted in this area when endogenous CHO stores are depleted. PURPOSE: The purpose of this study was to assess the effects of a CHO mouth rinse on endurance-trained cyclists during a 30-kilometer time trial in depleted vs. non-depleted glycogen states. METHODS: 5 endurance-trained men (28±7 years; 179.32±8.15 cm; 68.65±8.16 kg; 54.00±5.95 mL/kg/min VO2 max) participated in a repeated measures crossover study using a CHO mouth rinse (6.4% malto-dextrin) or placebo. RESULTS: Subjects completed the time trial faster (p=0.033) in the non-depleted state, despite reporting lower RPE (p=0.010). There was no significant treatment effect, but subjects completed the time trial 20 seconds faster in the depleted CHO mouth rinse condition. Power output was significantly higher (p=0.010) in non-depleted than depleted conditions; no significant treatment effect was observed. RER was significantly higher (p=0.001) in the non-depleted state; no significant differences were observed between mouth rinses. A mouth rinse segment interaction (p=0.028) was observed for heart rate in the last 6k for the CHO mouth rinse condition, indicating a possible increase in effort over the last segment of the time trial. CONCLUSION: CHO mouth rinse may be a valuable tool for performance enhancement in subop-timal nutrition states. Further, the results supported current knowledge regarding performance in depleted and non-depleted glycogen states.

I. Introduction

A. Background

Carbohydrate (CHO) supplementation and high muscle glycogen stores have been shown to improve athletic performance, particularly in prolonged events where muscle glycogen becomes depleted. A study by Bergström and colleagues investigating the relationship between muscle glycogen and physical performance found that the muscle glycogen content of the working muscles was a key determinant in the ability to perform long-duration exercise (Bergström et al. 1967). Further, Hargreaves and colleagues found that CHO supplementation during prolonged exercise decreased the depletion of intramuscular glycogen stores, increased blood glucose levels, and produced faster time trial performance (Hargreaves et al. 1984).

However, the effects of exogenous CHO in events lasting up to one hour in duration are more ambiguous due to limitations in absorption and the use of blood glucose for energy. In these shorter events, exogenous CHO makes a minimal contribution to the total CHO oxidation rate (Chambers, Bridge, & Jones 2009), as it is estimated that only 5-15 grams of exogenous CHO is oxidized during the first hour of exercise (Carter, Jeukendrup, Mann, & Jones 2004). Numerous studies have demonstrated the lack of influence that CHO ingestion has on muscle metabolism in shorter duration events (Rollo, Williams, Gant, & Nute 2008).

Despite this general conclusion, there have been studies showing that CHO ingestion can improve exercise performance in shorter duration (<1 hour), higher intensity (>75% of VO2max) tasks (Carter et al. 2004). Carter et al. (2004) investigated the mechanisms behind this performance improvement by utilizing glucose infusion rather than ingestion; in effect bypassing oral CHO receptors and eliminating variability in absorption rates among subjects. No performance differences were found in the 1-hour cycling time trial when the subjects received a placebo infusion versus a CHO infusion, indicating that an increased availability of glucose had no effect on exercise in which endogenous CHO stores were not limiting (Carter et al. 2004). This study suggests that the performance improvements seen with CHO ingestion in shorter-duration exercise are not due to the observed increase in blood glucose levels, but some other mechanism.

With this in mind, researchers have begun to investigate the potential performance benefits of a CHO mouth rinse. This method involves rinsing the mouth with a CHO solution and subsequently expelling it, thus eliminating the potential for CHO oxidation (Carter et al. 2004). The rationale behind this method is based on the "central effect." The oral CHO receptors that are activated by the presence of the CHO solution may send signals that initiate a positive central response in the brain. This model hypothesizes that the positive central response activated by oral afferents could potentially override the negative physical, metabolic, and thermal afferent signals coming from peripheral receptors. Generally, these negative afferent signals contribute to central fatigue and lead to a reduction in motor output; thus, by counteracting various negative afferent signals, these positive signals can aid in maintaining or improving performance (Carter et al. 2004).

Work by Chambers (2009) using fMRI images indicated that oral exposure to CHO activated reward and motor control regions in the brain, including the orbitofrontal cortex, striatum, and insula/frontal operculum. These findings are cited as possible reasons for exercise performance improvement after exposure to CHO (Chambers et al. 2009). Similarly, a study using a 30-minute treadmill time-trial found that subjects reported enhanced feelings of pleasure during the first 5 minutes of running when mouth rinsing with CHO (Rollo et al. 2008).

Numerous studies have been conducted to examine the effectiveness of the CHO mouthwash, with the majority involving cycling time trials. A systematic review found that 8 of the 11 stud-ies reviewed reported an increase in performance (ranging from 1.5% to 11.59%) with the use of a CHO mouth rinse in moderate to high-intensity exercise (65% of VO2max for 1 hr). The review provided further evidence that the effects of the CHO mouth rinse are neural rather than meta-bolic, as the CHO mouth rinse was not

associated with any changes in blood glucose levels (Silva, Maria, Amorim, Sta-this, Leandro, & Lima-Silva 2014). This review also suggests that the positive effect of the CHO mouth rinse is accen-tuated when muscle and liver glycogen stores are reduced (Silva et al. 2014). Fares & Kayser (2011) investigated the effect of CHO mouth rinse on cycling performance in pre and postprandial states, using non-athletic male subjects. While performance improvements were shown in both fasted and non-fasted subjects with the use of a mouth rinse, greater improvements were found in the fasted subjects (Fares & Kayser 2011). It is hypothesized that the effects of the mouthwash may be more prominent in a fasted state due to increased oral sensitivity of the receptors (Silvia et al. 2014). Given that glycogen depletion is a common cause of fatigue during endurance performance, it would be of value to further investigate the effectiveness of a mount rinse when CHO availability is limited.

B. Purpose

The purpose of this study was to assess the effects of a CHO mouth rinse in endurance-trained cyclists completing a 30-kilometer time trial in depleted vs. non- depleted glycogen states.

C. Hypotheses

It was hypothesized that the CHO mouth rinse would have a greater effect on cyclists in the depleted state compared to the non-depleted state, as demonstrated by a faster time to completion in a cycling time trial. Additionally, it is hypothesized that the presence of the CHO mouth rinse will not be associated with an increase in blood glucose levels or rates of CHO oxidation during the time trials.

D. Significance

This investigation of CHO mouth rinse is warranted because it could provide valuable information regarding the mechanisms and power of the central effect hypothesis. Additionally, the use of a CHO mouth rinse could provide avenues for performance improvements in competitive athletes as an alternative to CHO consumption, especially in those who experience GI distress from the ingestion of CHO during exercise.

II. Methods

A. Participants

Five endurance-trained athletes between the ages of 18 and 60 were selected to participate in this study (Table 1). Their classification as endurance-trained was verified with an activity questionnaire and a maximal aerobic capacity (VO2max) test. For the maximal aerobic capacity test, values of 50 ml/kg/min or greater were accepted under the classification of endurance-trained (Wilmore & Costill 1999). These participants were recruited from the local Fort Worth cycling and triathlon communities via word-of-mouth, email, and other electronic postings. Additionally, medical history questionnaires were administered to all potential subjects in order to exclude any participants who were unable to complete the task or were at risk for harm due to pre-existing health conditions. Exclusions (in addition to injury) were determined with the utilization of the ACSM guidelines.

	Mean	Minimum	Maximum
Age	27.80±7.09	22	36
Height (cm)	179.32±8.15	170.18	190.15
Weight (kg)	68.65±8.16	59.32	78.00
BMI	21.47±2.01	18.70	23.90
VO_2 max (mL/kg/min)	54.00±5.95	47.31	60.23
VO_2 max (L/min)	3.68±0.14	3.87	3.68

Table 1: Participant Demographics (n = 5)

B. Experimental Design

Each subject that was selected to participate in the study underwent four trials using a repeated measures design. The trials were administered in a randomized order. These trials consisted of:

1. A time trial in which the subject was in a non-depleted glycogen state and received a placebo mouthwash solution (ND-PLA);

2. A time trial in which the subject was in a non-depleted glycogen state and received a CHO mouthwash solution (ND-CHO);

3. A time trial in which the subject was in a depleted glycogen state and received a placebo mouthwash solution (D-PLA); and

4. A time trial in which the subject was in a depleted glycogen state and received a CHO mouth wash solution (D-CHO).

C. Experimental Procedures

On their first visit, potential subjects underwent preliminary testing to determine their suitability for inclusion in this study. During this visit, they completed the necessary paperwork (medical history, activity questionnaire, and in-formed consent). Following this, each subject completed a test for maximal oxygen uptake (VO2max) on a standard cycle ergometer (Velotron). This test involved a series of two and three-minute stages in which the wattage was systematically increased until the subject reached a point of voluntary termination, an inability to maintain proper cadence, age-predict-ed maximal heart rate, an RER greater than 1.1, or a blood lactate value of 8.0 mmol/L or higher. During this test, heart rate and respiratory exchange data was collected using an open circuit automated gas analysis system.

After this preliminary testing, participants had the opportunity to practice using the stationary cycle ergometer on the pre-set 30-kilometer time trial course. This ensured that no learning curve took place during the actual experimen-tal trials. During this visit, subjects were also familiarized with the testing protocol. Thus, they were informed at which points during the time trial they would be asked to rinse with the mouthwash solution, as well as how to conduct the mouth rinsing, and how and when they would be asked to provide their ratings of perceived exertion. The experimental treatments were randomly assigned and separated by a minimum of one week. Subjects abstained from caffeine, alcohol, tobacco, and exercise in the 48 hours prior to each visit. Each participant was asked to report at the same time of day for all of their individual trials in order to minimize the influence of diurnal variation, pre-trial meal variability, and other schedule-related extraneous factors.

All four experimental treatments were preceded by a glycogen-depletion exercise protocol. In this protocol, subjects cycled on the Velotron cycle ergometer for 60 minutes at 70% of their VO2max (calculated using the values obtained from the first visit). Immediately upon completion of the 60-minute ride, they completed six, 11- minute sprints, cycling at 120% of their VO2max with at least a 1-minute rest period in between each. For the next 24 hours following the glycogen depletion exercise, the subjects either consumed a diet consisting of 5% CHO (approximately 30-50 g CHO) or 75% CHO (approximately 600g CHO), depending on the experimental treatment. A diet recall was requested in order to ensure adherence to the macronutrient protocol. Upon completion of this 24-hour low or high-CHO period, subjects again reported to the lab to undergo the time trial.

The time trial consisted of a programmed 30-kilometer ride on the Velotron cycle ergometer that remained constant for each time trial. The course included simulated terrain, including climbing and descending hills, and flat stretches. Prior to each pre-trial warm-up, a blood sample was collected from each subject. Each trial procedure involved a 5-minute, self-selected warm-up period on the cycle ergometer, which was held constant for all subsequent trials. After this warm-up period, the time trial began. Subjects were asked to complete the 30-kilometer ride as fast as possible. During the 3rd, 9th, 15th, 21st, and 27th kilometer a respiratory gas exchange analysis was conducted by having the sub-ject breathe through a gas collection mouthpiece for three minutes. Every 3 kilometers, subjects provided their rating of perceived exertion and heart rate data were collected. Immediately following each 30-kilometer time trial, another blood sample was collected.

All experimental trials involved the implementation of a mouth rinse during the 30-kilometer time trial. The mouth rinse procedure involved a double-blind protocol. The subjects were asked to rinse with 25 mL of the solution, either 6.4% maltodextrin (ND-CHO & D-CHO) or a placebo (ND-PLA & D-PLA), for 10 seconds at the 6th, 12th, 18th, and 24th kilometers during the time trial. The placebo solution had no nutrient content of any kind. The CHO and placebo solutions were matched as closely as possible for taste, color , and consistency. The solution was administered to the subject orally with a plastic cup. After the 10 seconds of rinsing, the subject was asked to expel the solution back into the plastic cup. Pre-weight and post-weight measurements of the solution were used to determine if the subject ingest-ed any solution. The subjects continued to cycle during these mouth rinse periods, as the time trial was continuous. The respiratory gas exchange data were reduced to provide rates of CHO oxidation, and the blood samples were analyzed for glucose levels. Blood samples were analyzed using spectrophotometer readings. Increases in NADH concentration is directly proportional to glucose concentration and can be measured at 340 nm. This reaction was catalyzed by combining hexokinase with the blood samples.

D. Statistical Analysis

A two-factor condition by time repeated measures ANOVA was used for the statistical analysis of the majority of the dependent measures. The condition factor had four levels, which included depleted vs. non-depletedCHO status and CHO mouth rinse vs. placebo. The time factor had varying levels depending on the frequency of sampling (a minimum of two levels for pre and post, and up to five levels for variables collected during the time trial). A one-factor ANOVA with repeated measures was used for variables collected a single time for each trial. A Bonferroni post-hoc analysis was used

in order to isolate statistically significant differences in the dependent vari-ables of interest: total time to completion, power output, respiratory exchange ratio (RER), heart rate, and rating of perceived exertion (RPE). A p-value of <0.05 was used to determine statistical significance.

III. Results

Analysis of the depletion bout data revealed no statistically significant differences in percentage of maximal aerobic capacity, average heart rate, or average rating of perceived exertion between conditions (Table 2).

	ND CHO	ND Placebo	D CHO	D Placebo	P Value
VO$_2$ (% max)	72.79±6.28	72.77±8.12	72.76±4.24	73.84±3.54	.625
Avg. HR (bpm)	164.22±4.29	157.96±14.35	159.75±21.89	161.32±17.14	.445
Avg. RPE	14.8±1.30	14.3±1.68	14.5±51.5	14.0±1.22	.989

Table 2: Depletion Bout Cardiorespiratory and Subjective Data

Similarly, analysis of the participants' diet revealed no significant differences in total caloric consumption across all four experimental conditions (p=0.205). As intended, there was a significant difference in percentage of total calories from CHO (p<0.001) and grams of CHO consumption per kilogram of body weight (p<0.001) between the depleted and non-depleted glycogen conditions (D-PLA and D-CHO vs. ND-PLA and ND-CHO). (Table 3).

	ND CHO	ND Placebo	D CHO	D Placebo	P Value*
Energy consumption (kcal)	2978±536	2826±710	2308±401	2546±224	.205
Carbohydrate (% of total kcal)	65.2±10.4	70.0±7.9	5.2±1.5	6.2±1.8	<.001
Carbohydrate (g/kg)	7.14±0.82	7.26±1.72	0.44±0.06	0.58±0.12	<.001

Table 3: Diet Analysis: Caloric and CHO Content

Analysis of the total time to completion of the 30-kilometer cycling time trial between conditions re-vealed a diet main effect (Fig. 1). In the ND condition, participants cycled significantly faster than in the D condition (p=0.033). No significant difference was observed between CHO and PLA mouth rinse, though in the CHO mouth rinse condition participants cycled 20 seconds faster on average than in the PLA condition.
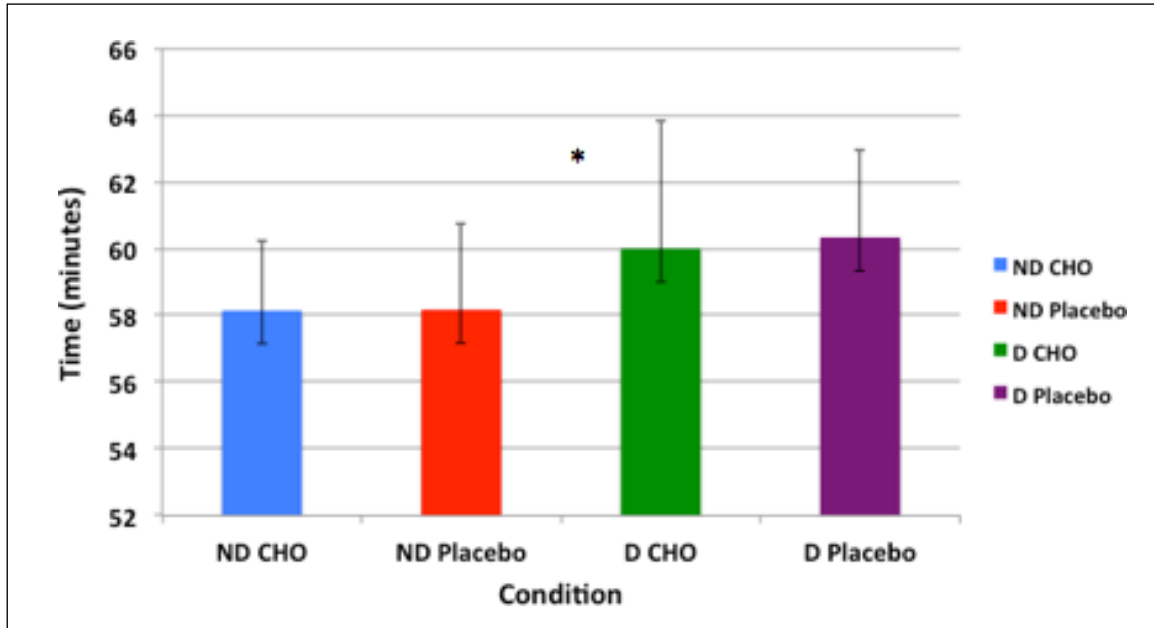
*Figure 1: Time Trial Time to Completion. * Diet main effect, p=0.033*

Similarly, analysis of power output revealed a diet main effect (Fig. 2a). Participants in the ND condition produced significantly greater power output than when in the D condition (p=0.010). Additionally, a segment main effect was observed in the last 6 kilometers of the time trial (Fig. 2b). Participants produced a significantly greater power output over the last 6 kilometers in all conditions (p=0.010).
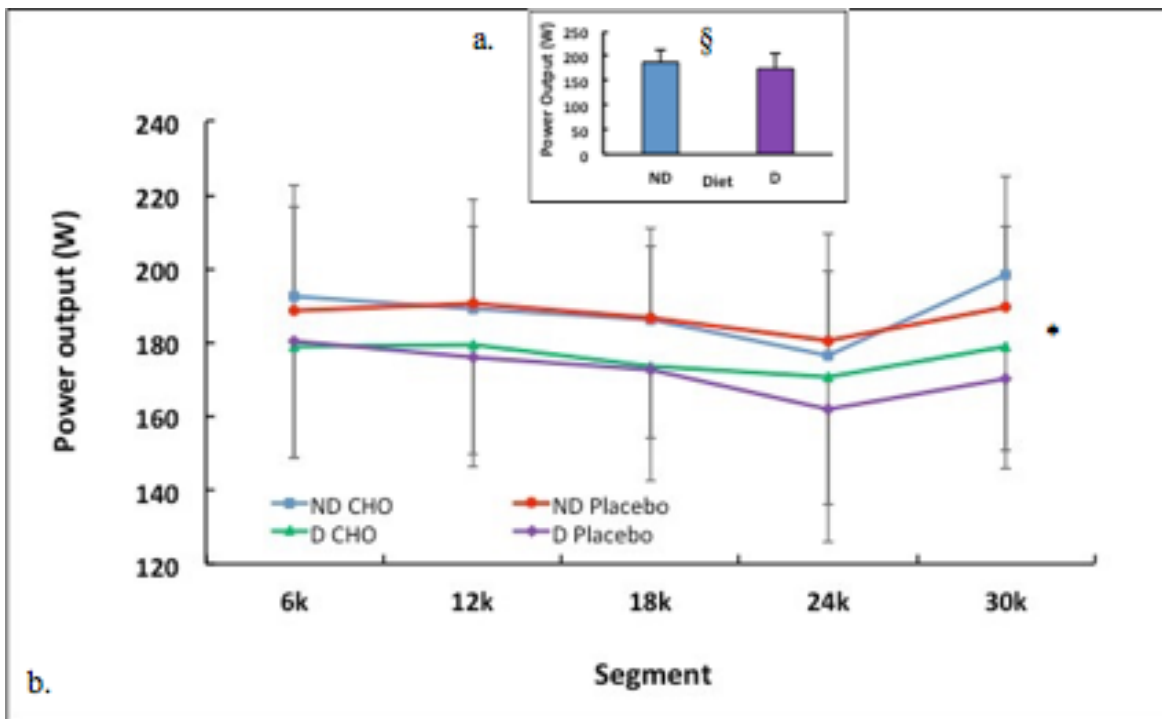

*Figure 2a:Time Trial Average power output. * Diet main effect, p=0.010*
Figure 2b: Time Trial Segmental power output. § Segment main effect, p=0.010

A diet main effect was observed for average respiratory exchange ratio, with the ND condition displaying a significantly higher (p=0.001) RER than the D condition (Fig. 3a). No segment main effects were observed (Fig. 3b).
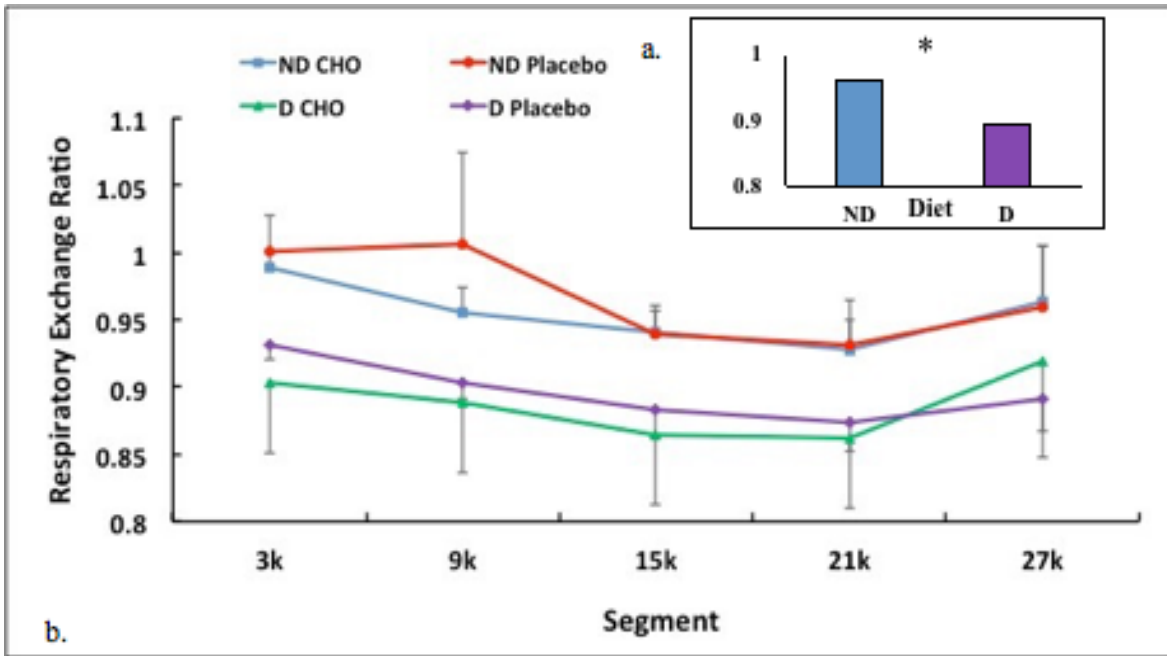

*Figure 3a: Time Trial Average respiratory exchange ratio. *Diet main effect, p=0.001*
Figure 3b: Time Trial Segmental respiratory exchange ratio. No effects.

Heart rate data showed a segment interaction over the last 6 kilometers between mouth rinse conditions (Fig. 4). In the D condition, participants had a significantly higher heart rate than in the ND condition over the last 6 kilometers (p=0.028).
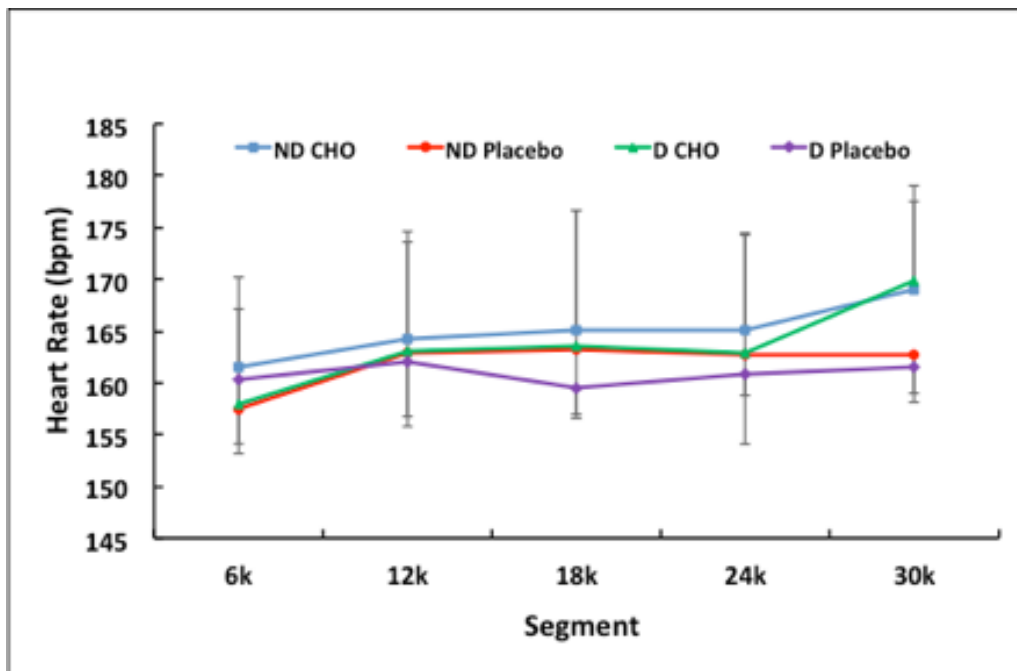

*Figure 4: Time Trial Segmental Heart Rate Data. * = Mouth Rinse * Segment Interaction, p = 0.028*

A diet main effect was observed with rating of perceived exertion (Fig. 5a). In the D condition, participants had a significantly higher RPE than when in the ND condition (p=0.010). Additionally, a segment main effect was observed with participants reporting a significantly greater RPE (p<0.001) over the last 6 kilometers (Fig. 5b).
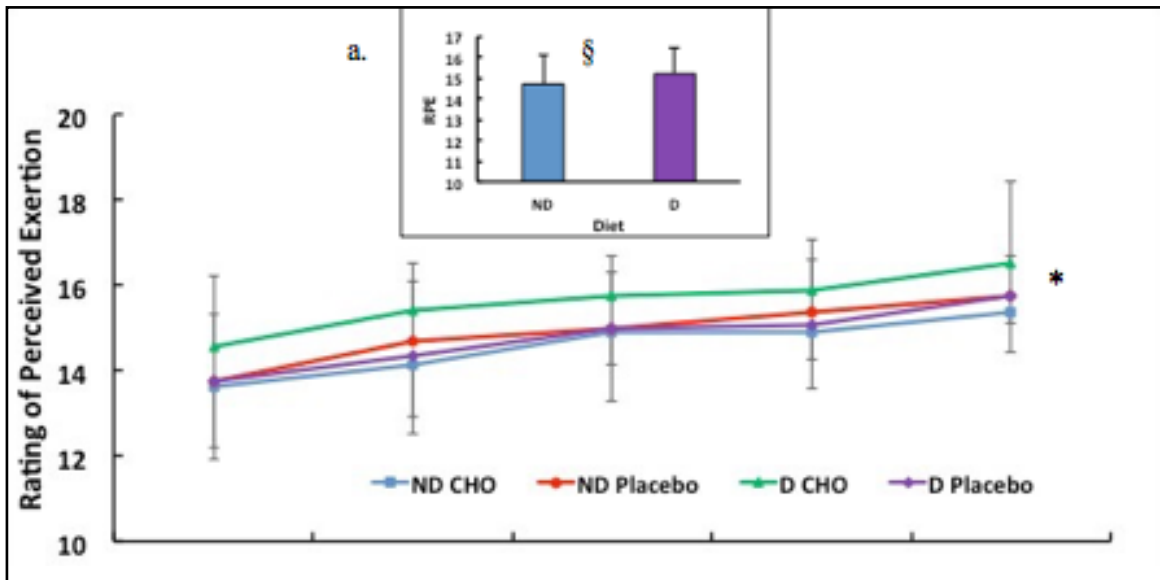

Figure 5a: Time Trial Average Rating of Perceived Exertion. § Diet main effect, p=0.010

Figure 5b: Time Trial Segmental Rating of Perceived Exertion. * Segment main effect, p<0.001

IV. Discussion

As expected, diet-based main effects were observed in most variables, including total time to completion, average power output, average respiratory exchange ratio, and average rating of perceived exertion. Sufficient (non-depleted) glycogen stores enabled participants to cycle faster with greater power and lower perceived exertion. This difference in glycogen storage was supported by the diet-based main effect observed in respiratory exchange ratio as participants in the non-depleted condition had significantly greater average RER values than when they were in the depleted condition. An RER value of 1.0 indicates 100% CHO oxidation, while an RER of 0.7 indicates 100% fat oxidation; thus, while depleted, subjects were relying more heavily on the use of fat as an energy source (Zuntz 1901). These data support existing literature on the benefits of adequate glycogen storage on exercise performance (Bergström et al. 1967).

The main purpose of this study was to investigate the effects of the CHO mouth rinse on the 30-kilometer cycling time trial performance. It was hypothesized that the CHO mouth rinse would have a greater effect on athletes in the depleted state than in the non-depleted state, as demonstrated by a faster time to completion in a cycling time trial. The hypothesis was rejected, as no significant difference was found in total time to completion between the CHO and PLA mouth rinse conditions. However, participants cycled an average of 20 seconds faster in the CHO mouth rinse condition than in the PLA mouth rinse condition. While this difference was not significant, the study was limited by a small sample size. It is possible that increasing the number of participants involved in the study may provide greater insight into the possible beneficial effects of the CHO mouth rinse on cycling performance. Additionally, an analysis of biking world championships from the past eighteen years, including the UCI World Championships Men's Time Trial event and Olympics Men's Time Trial event revealed that, on average, approximately 20 seconds separated the third and fourth place finishers. This data provides evidence that although the 20 second difference between the CHO and PLA conditions in a fasted state were not statistically significant, the mouth rinse may provide tangible benefits.

   The only significant effect observed between the CHO and PLA mouth rinse conditions was a higher heart rate over the last 6 kilometers of the time trial in the CHO mouth rinse condition. It is possible that, while the usage of the CHO mouth rinse did not translate into faster total completion times, the participants were able to exert a greater effort over the last 6 kilometers of the time trial. Perhaps with a greater sample size or longer time trial distance, this increase in effort would be correlated with faster completion times.

   In terms of methodology, the study was successful in creating a consistent depletion protocol. The data for the de-pletion bouts were consistent across all four conditions. Therefore, differences in time trial performance should not be attributed to varying intensity of depletion bouts. Additionally, the participants adhered to the diet protocol in a means that was effective for the goals of the study. No significant differences in total caloric intake were observed over the 4 conditions. Thus, the differences between the ND and D conditions could be attributed solely to CHO content. Further, the CHO content between the ND and D conditions was statistically significant. Though the subjects did not reach the goal values of 75% and 5% for high and low CHO, respectively, they closely approximated these values and thus achieved the goal of the depletion and diet manipulation protocol.

   The analysis completed on the blood samples collected both before and after each of the time trials revealed no significant differences in blood glucose levels. If participants had ingested any of the mouth rinse during the trials, we would have expected to see an increase in blood glucose levels from the pre-time trial sample to the post time trial sample. Since such an increase was not seen, we can assume that participants did not ingest a significant amount of the mouth rinse, meaning that any improvements via the mouth rinse can likely be attributed to neural effects via the oral CHO receptors, as described in previous studies (Silvia et al. 2014).

   In general, our results did not support the existing data that cites the benefits of a CHO mouth rinse when compared to a PLA mouth rinse on time trial performance (Silva, Maria, Amorim, Stathis, Leandro, & Lima-Silva 2014). However, the studies that investigate mouth rinse effects in depleted and non-depleted glycogen states are sparse. Further studies on this topic should involve a larger participant pool and more stringent control over potential confounding variables such as sleep, training, and other outside stressors.

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